

APPENDIX I

REVISIONS OF THE TITLE PURSUANT TO REVISED RULE § 1.121

Pursuant to Revised Rule § 1.121, the revisions of the title are detailed as follows:

Please amend the title to read as follows:

Methods for the Storage and Synthesis of Nucleic Acids using a Solid Support

REVISIONS OF THE SPECIFICATION PURSUANT TO REVISED RULE § 1.121

Pursuant to Revised Rule § 1.121, the revisions of the specification are detailed as follows:

Please replace the paragraph at page 1, lines 5-17, with the following paragraph:

The present application claims the benefit of U.S. Provisional Application No. 60/175,307, filed January 10, 2000, the disclosure of which is incorporated herein by reference in its entirety. [The present application is also related to inventions described in United States patent application serial number 09/054,485, filed April 3, 1998, and is related to the continuing application based thereon filed December 23, 1999, and is related to United States patent application serial number 09/076,115, filed May 12, 1998, and is related to United States patent application serial number 09/354,664, filed July 16, 1999, and is related to United States provisional patent application serial number 60/046,219, filed May 12, 1997, and is related to United States provisional application serial number 60/042,629, filed April

3,1997, and is related to United States provisional application serial number 60/122,395, filed March 2, 1999 the disclosures of which are specifically incorporated herein by reference.]

Please replace the title of the table at page 27, line 8, with the following:

Table 1. Primer Sequences used. (SEQ ID NOS 1-12, respectively in order of appearance)

Please replace the paragraph at page 28, line 23, to page 29, line 17, with the following paragraph:

The results of the amplification of nucleic acids stored on solid supports are shown in Figures 2-4. Figure 2 shows the results of the amplification of nucleic acids from HeLa cells. Eluted RNA was precipitated from washes taken from 2-mm punches of HeLa cell samples stored at -20° and -70°C for 1 year as described above. The amplification targets were as follows: Panel A; a 626 bp sequence from b-actin mRNA was amplified using the following thermocycling conditions: 94°C for 1 min, followed by 40 cycles of 94°C for 30 s; 60°C for 30 s and 72°C for 1.5 min; forward and reverse primer sequences were 5'CCTCGCCTTTGCCGATCC3' (SEQ ID NO: 9) and 5'GGATCTTCATGAGGTAGTCAGTC3' (SEQ ID NO: 10), respectively. Panel B; a 1.08-kb sequence of RPA (replication protein A) mRNA was amplified using the following thermocycling conditions: 94°C for 1 min, followed by 40 cycles of 94°C for 30 s; 55°C for 30 s and 72°C for 1.5 min; forward and reverse primer sequences were 5'CAAGATGTGGAACAGTGGATTTC3' (SEQ ID NO: 7) and 5'CATCTATCTTGATGTTGTAACAAGC3' (SEQ ID NO: 8), respectively. and Panel C: a 5.76-kb sequence of a clathrin-like protein (D21260) mRNA was amplified using the following thermocycling conditions: 94°C for 1 min, followed by 35 cycles of 94°C for 20 s; 60°C for 30 s and 68°C for 7 min; forward and reverse primer sequences were 5'CCCAGTGACAGGAGGAGACCATA3' (SEQ ID NO: 11) and 5'ATCCTGTGCTTTTTCTGTGGGAC3' (SEQ ID NO: 12), respectively. For Panels A and B, Lanes 1-3 and 4-6 are from samples stored at -20°C and -70°C, respectively subsequent to sample application onto FTA® GeneCards, whereas lane 7 is a negative control where

SUPERSCRIPT II RT was omitted from the RT reaction. Lanes labeled M are a 1 kb ladder size markers. For Panel C, lanes 1, positive control, HeLa RNA, Lanes-2 and 3 are from samples stored at -70°C subsequent to sample application onto FTA® GeneCards, whereas lane 4 is the negative control.

Please replace the paragraph at page 31, lines 13-29, with the following paragraph:

Poly(A+)RNA was directly isolated from 2.25×10^6 BHK-21 cells stored on FTA® paper as described above except that the biotinylated oligonucleotide(dT) had special adapter sequences necessary for library construction. The primer includes a *Not* I recognition site and has the sequence (Biotin)₄ GACTAGTTCTAGAT CGCGAGCGG CCGCCCTTTTT TTTTTTTTTTTT TTTTTTTT (SEQ ID NO: 13); (see WO 98/51699 and United States application serial number 09/076,115). As a positive control, poly(A+) RNA was isolated total RNA prepared by TRIzol reagent from the same number of cells. Double-stranded cDNA was made and cloned into plasmid vectors as described in WO 98/51699 and United States application serial number 09/076,115. The number of primary clones obtained from the poly(A+)RNA was the same whether the mRNA was isolated directly from FTA® or from TRIzol-purified total RNA. The average insert size of the libraries was determined by colony PCR using primers to the plasmid vector. The average insert size for the FTA®-derived material was greater than that for the library constructed from the positive control poly(A+)RNA, 1000bp vs 600 bp. This indicates that cDNA libraries of good quality can be made from mRNA isolated directly from samples stored on FTA®.

The remaining amendment to the specification was an addition, and a marked copy is not required.

REVISIONS OF THE CLAIMS PURSUANT TO REVISED RULE § 1.121

Pursuant to Revised Rule § 1.121, the revisions of the claims are detailed as follows:

Please amend claims 1-7 to read as follows:

--1 (amended). A method to produce one or more cDNA molecules comprising:

- (a) contacting a sample comprising an [one or more] mRNA template[s] with a solid medium, wherein the solid medium comprises a matrix; [support; and]
- (b) sorbing at least a portion of the mRNA template to the solid medium; and
- (c) contacting the [said] template[s] with one or more reverse transcriptases under conditions sufficient to synthesize one or more cDNA molecules complementary to all or a portion of the [said] templates.

2 (amended). The method of claim 1, wherein the [said] cDNA is a cDNA library.

3 (amended). The method of claim 1, wherein the [said] mRNA template is removed from the solid medium [said support] prior to the [said] cDNA synthesis.

4 (amended). The method of claim 1, wherein the [said] cDNA is double-stranded.

5 (amended). The method of claim 1, further comprising:

- (d) amplifying the [said] cDNA.

6 (amended). A method for storing an mRNA [RNA] molecule, comprising:

- (a) contacting a cell comprising an mRNA [RNA] molecule to be stored with a solid medium, wherein the solid medium comprises a matrix containing a composition for substantially inhibiting degradation of the mRNA molecule [support]; and
- (b) drying the cell and the solid medium [support].

7 (amended). The method of claim 6, wherein the composition comprises:

- (a) a weak base;
- (b) a chelating agent; and
- (c) an anionic detergent or surfactant

[solid support is FTA® paper].--